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# Conditioned taste aversion is reduced in rats with a history of lithium/pilocarpine-induced limbic seizures

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Limbic seizures were evoked in rats by single subcutaneous injections of lithium and pilocarpine that are known to elicit severe damage to gustatory-affective centers in the brain. About two months later 12 of these rats and 12 control rats that received either lithium or pilocarpine (no seizures) were given free access to 10% sucrose solutions and then injected with 0.15 M LiCl (10 ml/kg). Whereas the control rats demonstrated the expected conditioned taste aversion (CTA) to later presentations of sucrose during single bottle drinking tests, the rats that had been seizured demonstrated a significant attenuation in CTA

Systemic or peripheral injections of cholinergic compounds are known to produce neuronal necrosis within limbic and thalamic structures [1, 7]. Rats that survive limbic seizures that are evoked by single subcutaneous injections of lithium chloride (3 mequiv/kg) followed 4–24 h later by a single subcutaneous injection of pilocarpine (30 mg/kg) demonstrate total neuronal loss (cystic lesions) in the amygdala and entorhinal-pyriform cortices. Some but not all animals showed damage in the insular cortex [3]. Usually adult (over 100 days of age) rats die within 3 days of these seizures or show severe aphagia and adipsia. Recently, methods have been determined [4] to insure the survival (injection of 30 mg/kg of acepromazine between 0 and 1 h after the administration of pilocarpine) of these animals so that behavioral correlates of what appears to be progressive seizure-induced brain damage can be examined.

The most obvious hypothesis, in light of the pattern of seizure-induced brain damage, is that these rats should display a reduction in conditioned taste aversion (CTA). This phenomenon is a powerful example of one trial learning; it involves pairing of a novel gustatory stimulus (such as sucrose) with a dysaffective (usually nox-

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ious) stimulus [2]. Our hypothesis was based on the recent anatomical observations by Shipley [6] who also reviewed the existing information on taste-visceral pathways. Our second hypothesis was that the seizured animals (as a group) should demonstrate a greater variability in the display of CTA in light of the variable involvement of the insular cortices that follows lithium/pilocarpine-induced seizures [3].

A total of 12 seizured and 12 non-seizured male 250-day-old Wistar albino rats (500–600 g) were tested in 3 experimental blocks (4 seizured and 4 control rats per block) according to the procedures of Persinger and Fiss [5]. Two months before each rat in the first group had been given the epileptogenic treatment: a single injection (subcutaneously) of 3 mequiv/kg of lithium chloride followed 4 h later by a single injection (subcutaneously) of 30 mg/kg of pilocarpine. Limbic seizures ensued within about 30–40 min. One h after the injection of the pilocarpine (20–30 min after seizure onset), the rats were injected subcutaneously with 30 mg/kg of acepromazine, a procedure that was found to reduce later mortality from 95% to less than 5% in older animals [4]. Two days later the seizured rats were given a milk and bread diet in order to combat the typical aphagia and adipsia that follows these seizures, until they could return to the regular diet (rat chow and water) within about 7–21 days. The control rats were injected with either lithium (3 mequiv/kg) or pilocarpine (30 mg/kg); although there were no seizures, half the numbers of control rats (3 from the pilocarpine only and 3 from the lithium only subgroups) were exposed to the milk and bread diet. All animals were individually housed in wire cages and by the beginning of the CTA experiment were eating and drinking ad libitum.

The subjects were habituated to drinking fluids for 20 min once per day. After about 5–7 days of water availability during this time, they were given access to 10% sucrose; this was followed within 5–15 min by a single intraperitoneal injection of 0.15 M lithium chloride (10 ml/kg; 1.5 mequiv/kg). Following two more days of water option, the rats were exposed to the sucrose (only) solution on the test day. After another week of daily water presentations, the rats were given another pairing of 10% sucrose with the 10 ml/kg intraperitoneal injection of 0.15 M LiCl, another two days of water option and then a second test day when sucrose (only) was presented again. The total amount of water or sucrose consumed per session was measured by graduated drinking bottles to the near 1 ml. Further details can be found elsewhere [5].

The combined results of the three experiments can be seen in Fig. 1. The rats that had been seizured 2 months before demonstrated less conditioned taste aversion as noted by their greater consumption. The differences (all df = 1,22) between the two groups for the days on which sucrose was available were: first pairing day ($F = 1.46$, $P > 0.05$), first test day ($F = 7.38$, $P < 0.01$), second pairing day ($F = 17.12$, $P < 0.001$) and second test day ($F = 11.40$, $P < 0.001$). In addition the variance in the sucrose consumption was significantly ($P < 0.01$) greater for the seizured rats on the two test days (Bartlett Box = 6.84 and 8.11, respectively) but not on the pairing days. $\chi^2$-Analyses (4.32 and 11.40), respectively indicated that the group differences on the two test days were statistically significant ($P < 0.01$). There was no difference between the groups with respect to water consumption before pairing began.

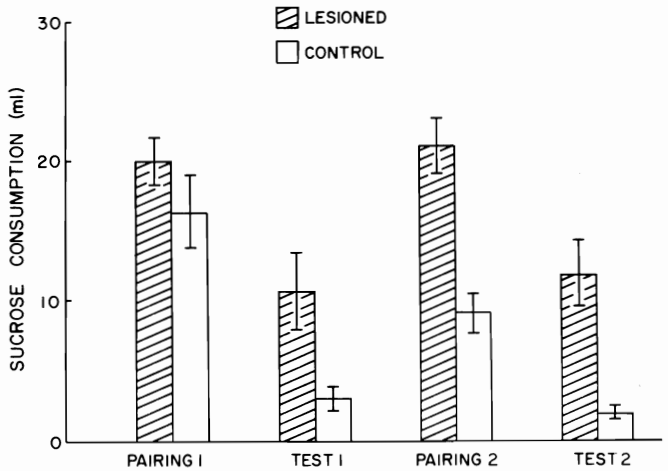


Fig. 1. Mean sucrose consumption (in ml) of rats (hatched bar, $n=12$) with a history of limbic seizures and of control rats (open bars, $n=12$). Vertical lines indicate S.E.M. The limbic seizures had been evoked by a single subcutaneous injection of 3 mequiv/kg of lithium chloride and 30 mg/kg of pilocarpine approximately two months before this experiment. Pairing days refer to days where the 10% sucrose solution was followed by lithium chloride while testing days occurred on the third days after the pairings.

MANOVA (multivariate analysis of variance) demonstrated a significant difference between groups ($F_{1,22}=13.53$, $P=0.001$). There were significant day differences ($F_{3,66}=34.92$, $P<.001$), primarily between pairing and test days, and a significant group by day interaction ($F_{3,66}=3.17$, $P=0.03$). A posteriori correlated $t$-tests indicated no significant differences in sucrose consumption (all df$=11$) between the two pairing days ($t=0.59$) or test days ($t=0.51$) for the seizured group but significant differences between both pairs of pairing and test days ($t=4.21$; 4.59). On the other hand, the control rats (all df$=11$) demonstrated significantly less sucrose consumption on the second pairing day compared to the first ($t=3.35$, $P<0.01$). There were highly significant ($P<0.001$) differences in sucrose consumption between both pairs of pairing and test days ($t=5.36$, 6.52). The absolute values of sucrose consumption for both the pairing and testing days for the control animals are comparable to our previous study [5]. A posteriori $t$-tests indicated no significant ($P\geqq 0.05$) differences in sucrose consumption between the 6 control rats (3 pilocarpine only and 3 lithium only) that had received the special milk and bread diet and the 6 control rats (3 pilocarpine only and 3 lithium only) that had not.

We interpret these data to indicate that the specific brain damage to amygdaloid, entorrhinal/pyriform and selected thalamic tissue that occurs within 50 days following limbic seizures produced by single subcutaneous injections of lithium and pilocarpine interferes with the gustatory-visceral pathways. Consequently both the acquisition and the memory (as indicated by the reduced CTA during the second pairing day) of a substance that is associated with dysaffective consequences may have been impaired. The specificity of this pathway and the involvement of the insular cortex [6] are suggested by both the disruption of CTA and the higher variability of the su-

crose consumptions on the testing days but not the training (pairing) days displayed by the seizured rats. The latter observation was expected on the bases of the variable occurrence of lesions within the insular cortices of seizured animals from other studies [3]. The seizure model used in this study may be useful for studying not only CTA but also the behavioral correlates of progressive, seizure-induced brain damage.

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